EFFECT OF MONOVALENT CATIONS ON CALCIUM TRANSPORT IN MITOCHONDRIA

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Cardiac glycosides and other factors influencing Na,K-ATPase activity and the ratio between intracellular Na^+ and K^+ concentrations lead to changes in cardiac and smooth muscle tone [5, 9], secretion of hormones and mediators [6, 10], and sensitivity of tissues to the action of various hormones [11]. At the same time, interaction between actin and myosin, the level of secretory activity, and activity of enzymes of the cyclic nucleotide system are known to be regulated by the free intracellular Ca^{++} concentration [3, 13, 14]. Accordingly it can be tentatively suggested that in the cases mentioned above the influence of cardiac glycosides on tissue metabolism is mediated through a change in the intracellular distribution of Ca^{++} and, in particular, through a change in Ca^{++} accumulation by intracellular structures.

In the investigation described below the effect of the ratio between Na^+ and K^+ concentrations on Ca^{++} accumulation by isolated mitochondria of myocardium, brain, and adipose tissue, as the principal Ca-buffer factor in the cells of these tissues, was investigated [1, 12].

EXPERIMENTAL METHOD

Male Wistar rats weighing 150-180 g, aged 2-3 months, were used. The procedures for isolating mitochondria from myocardium, brain, and adipose tissue and also the characteristics of the membrane preparations obtained were described previously [1, 12, 15]. The method of determining incorporation of ^{45}Ca into isolated membrane fractions also was described previously [1]. The composition of the incubation medium is given in the captions to Figs. 1-4.

Reagents: NaCl, KCl, $CaCl_2$, succinic acid, Tris-HCl (from Merck, West Germany), ATP-Tris(from Sigma, USA), ruthenium red (from BDH, England), $^{45}CaCl_2$ (from the Radiochemical Centre, Amersham, England). The remaining reagents were from Soyuzreakhim, of the chemically pure grade.

EXPERIMENTAL RESULTS

The kinetics of Ca^{++} accumulation by myocardial mitochondria in the presence of ATP and succinate is shown in Fig. 1. Under these conditions, an equilibrium distribution of ^{45}Ca was established between the mitochondria and incubation medium after 20 min of incubation. The Ca-accumulating capacity of the mitochondria, i.e., the maximal amount of Ca^{++} accumulated by mitochondria in incubation medium of this composition, was evidently determined by the ratio between the velocities of inflow and outflow of the cation.

It will be clear from Fig. 2 that an increase in the NaCl concentration to 60 mM accompanied by a decrease in the KCl concentration from 120 to 60 mM leads to a significant decrease in the Ca-accumulating capacity of the myocardial and brain mitochondria. Differences in the Na^+ concentration required to obtain half the maximal value of the effect (7 and 30 mM for myocardial and brain mitochondria, respectively), incidentally, agreed with

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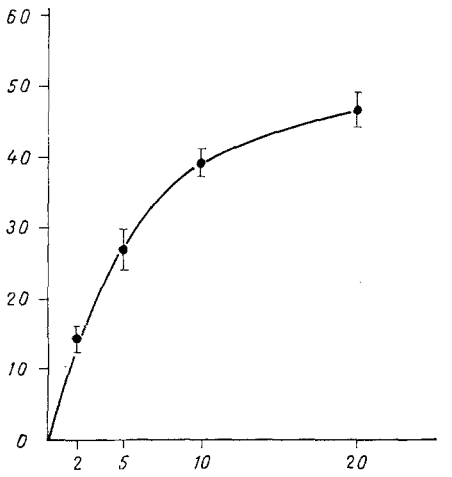

Fig. 1. Kinetics of Ca^{++} accumulation by myocardial mitochondria in incubation medium containing 50 mM Tris-HCl, pH 7.4; 120 mM KCl; 4 mM $MgCl_2$; 12 μM $CaCl_2$; 2 μCi/ml of $^{45}CaCl_2$; 4 mM ATP-Tris, and 10 mM succinate-Tris. Abscissa, incubation time (in min); ordinate, Ca-accumulating capacity (in moles/mg protein).

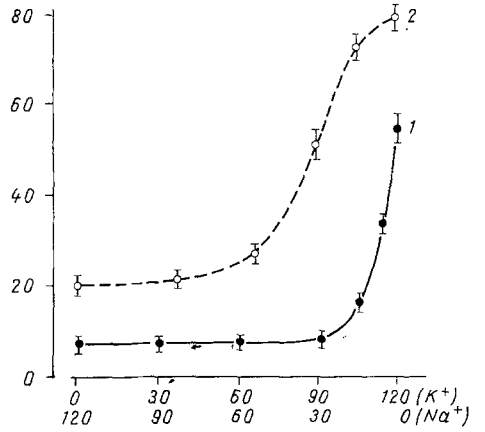

Fig. 2. Dependence of Ca-accumulating capacity of myocardial (1) and brain (2) mitochondria on ratio between Na^+ and K^+ concentrations in incubation medium containing 50 mM Tris-HCl, pH 7.4; 5 mM $MgCl_2$; 12 μM $CaCl_2$; 4 mM ATP-Tris, and 10 mM succinate-Tris. Abscissa, ratio between K^+ and Na^+ concentrations (in mM); ordinate, Ca-accumulating capacity (in nmoles/mg protein/20 min).

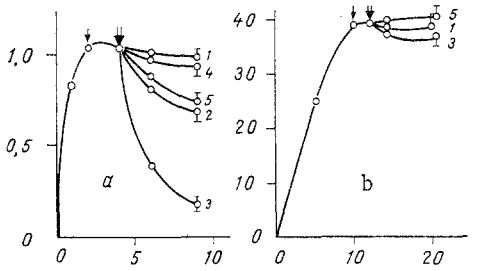

Fig. 3. Effect of monovalent cations on Ca^{++} liberation from mitochondria. Incubation medium: a) 50 mM Tris-HCl, pH 7.4; 250 mM sucrose, 5 mM $MgCl_2$, 12 μM $CaCl_2$, 2 μCi/ml $^{45}CaCl_2$, and 10 mM Tris-succinate; b) the same + 4 mM ATP-Tris. Single arrow — ruthenium red (2.5 μM) added, double arrow — NaCl (2 and 3) or KCl (4 and 5) added in concentration of 10 mM (2 and 4) or 40 mM (3 and 5). 1) Control (without addition of monovalent cations). Remainder of legend as in Fig. 1.

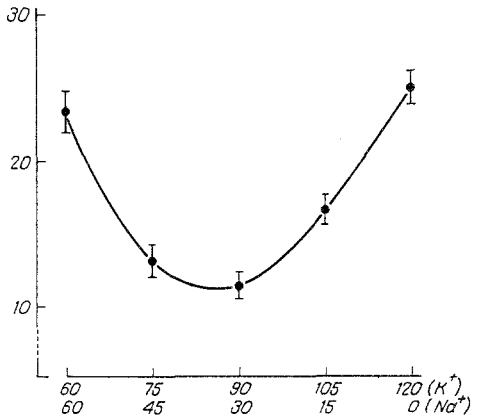

Fig. 4. Ca-accumulating capacity of mitochondria of adipose tissue as a function of ratio between Na^+ and K^+ concentrations in incubation medium. Composition of incubation medium and explanation of axes of coordinates given in caption to Fig. 2.

differences in the Na^+ concentrations in the cytoplasm of these tissues (8-15 and 30-40 mM respectively for myocardium and nerve tissue [4]). These observations indicate that the Na-dependent regulation of the Ca-capacity of mitochondria of excitable tissues *in vivo* is a possibility.

The cause of the decrease in the Ca-accumulating capacity of the mitochondria with an increase in the Na^+ concentration in the incubation medium could be slower inflow of Ca^{++} into and (or) faster outflow from the mitochondria. Data on the effect of monovalent cations on the electrochemical potential of the inner mitochondrial membrane, which determines the rate of Ca^{++} accumulation, are negative in character [2]. Meanwhile it was shown in 1974 that addition of Na^+ leads to the rapid outflow of ^{45}Ca from myocardial mitochondria previously loaded with isotope in the presence of succinate, and the further entry of Ca^{++} into which was retarded by the addition of ruthenium red [7, 8]. This effect was reproduced in the present experiments on the fraction of myocardial mitochondria (Fig. 3a). However, if the

mitochondria were loaded with ^{45}Ca in the presence not only of succinate, but also of ATP, subsequent addition of Na^+ did not lead to any significant release of Ca^{++} from the organelles (Fig. 3b).

The data given in Figs. 2 and 3a, b can be explained on the assumption that Ca^{++}-transport centers inside the mitochondria and the sites of the Na-Ca-carrier responsible for outflow of Ca^{++} are located close together on the inner membrane of the organelles, whereas the centers for formation of Ca-ATP-inorganic phosphate formation are distant from these sites and that the Ca^{++} present in these complexes plays no part in transmembrane exchange. The model put forward assumes that if ATP is present in the incubation medium, a Na-effect can be recorded only if the Ca^{++} inflow channels are uninhibited, which is in agreement with the experimental data described above.

It must be pointed out, however, that the mechanism of involvement of Na^+ in regulation of the Ca capacity of the mitochondria we have just examined may perhaps not be universal for mitochondria of all tissues. For instance, in the case of mitochondria of adipocytes, dependence of Ca-accumulating capacity on ratio between Na^+ and K^+ concentrations in the incubation medium is highly complex in character (Fig. 4): An increase in the Na^+ concentration up to 30-40 mM reduced Ca^{++} accumulation by half, but further replacement of K^+ by Na^+ restored the Ca capacity of the organelles to the original values. Explanation of the mechanism of regulation of the Ca-accumulating capacity of the mitochondria of unexcitable tissues by monovalent cations evidently requires additional experimental study.

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